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Effects of Intrathecal and Systemic Administration of Buspirone on Genital Reflexes and Mating Behavior in Male Rats

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MATHES, C. W., E. R. SMITH, B. R. POPA AND J. M. DAVIDSON. *Effects of intrathecal and systemic administration of buspirone on genital reflexes and mating behavior in male rats.* PHARMACOL BIOCHEM BEHAV **36**(1) 63–68, 1990.— Buspirone was studied to determine whether the detailed profile of male sexual behavior observed following treatment with the prototypical 5-HT_{1A} ligand, 8-OH-DPAT, can be generalized to other 5-HT_{1A} agonist drugs. Systemic and intrathecal (IT) routes of administration were compared. Like DPAT, significant reduction in intromission frequency followed IT infusion of buspirone (80–160 μg) as did intraperitoneal (IP) injection (1–4 mg/kg). IT doses of 80–160 μg and all IP doses significantly reduced ejaculation latency. Intercopulatory interval significantly decreased following IP buspirone but not after IT infusion although there were trends in that direction. All IP doses and 80 μg IT significantly shortened the postejaculatory interval. Buspirone inhibited erection and/or ejaculation in the ex copula reflex test. A decrease in percentage of rats displaying erections and ejaculation occurred following either route of administration. Ejaculation was significantly inhibited at the low IT dose of 40 μg. We conclude that buspirone affects sexual behavior very much like DPAT or other 5-HT_{1A} drugs, to the extent known. Sexual effects of buspirone were generally similar regardless of route of administration, but the effective doses were clearly lower with IT treatment.

Ejaculatory behavior Penile reflexes 5-HT_{1A} receptor agonists Ejaculation Copulatory plugs

CONSIDERABLE evidence has accumulated indicating an important modulatory role of the serotonin$_{1A}$ receptor (5-HT_{1A}) in certain aspects of the male rat's sexual behavior (2, 3, 11, 14, 15, 20, 23). The prototypical ligand used in these studies was 8-hydroxy-2-(di-*n*-propylamino)tetralin (DPAT) and the primary effect of this 5-HT_{1A} receptor agonist on mating behavior is lowering of the threshold of ejaculatory behavior (No. of intromissions to ejaculatory behavior). (It should be noted that "ejaculation" refers to seminal fluid expulsion from the urethra while "ejaculatory behavior" refers to the other *behavioral* events which naturally accompany ejaculation.) In addition DPAT also can stimulate the rate at which copulation occurs and facilitates postejaculatory sexual rearousal. RDS-127, a dopamine agonist with strong 5-HT_{1A} binding affinity (6,10) has a similarly marked facilitation of ejaculatory behavior, but differs in some respects from DPAT. RDS-127 dramatically reduces the ejaculatory behavior threshold but has no effect on the rate of intromissions (6–8). Furthermore RDS-127 stimulates ejaculation either in or ex copula (7, 8, 22) while DPAT inhibits ejaculation both in and ex copula (11, 17–19, 20).

In light of the apparent differences between the effects of these two drugs on sexual behavior, the present study sought to examine in some detail the effects of a previously unstudied potent 5-HT_{1A} receptor agonist, buspirone. We wished first to examine the generalizability of our hypothesis that 5-HT_{1A} receptor modulation is pivotal to the regulation of the ejaculatory behavior threshold. Second, we wished to determine if a locus of action of the sexual effects was the lumbosacral spinal cord as has been shown for DPAT (11).

METHOD

Animals

Sixty-two adult male Long-Evans rats (Simonsen Labs, Gilroy, CA), sexually experienced for mating behavior and genital reflexes, were utilized in these studies. Thirty-one rats received intrathecal (IT) catheters and were subsequently housed individu-

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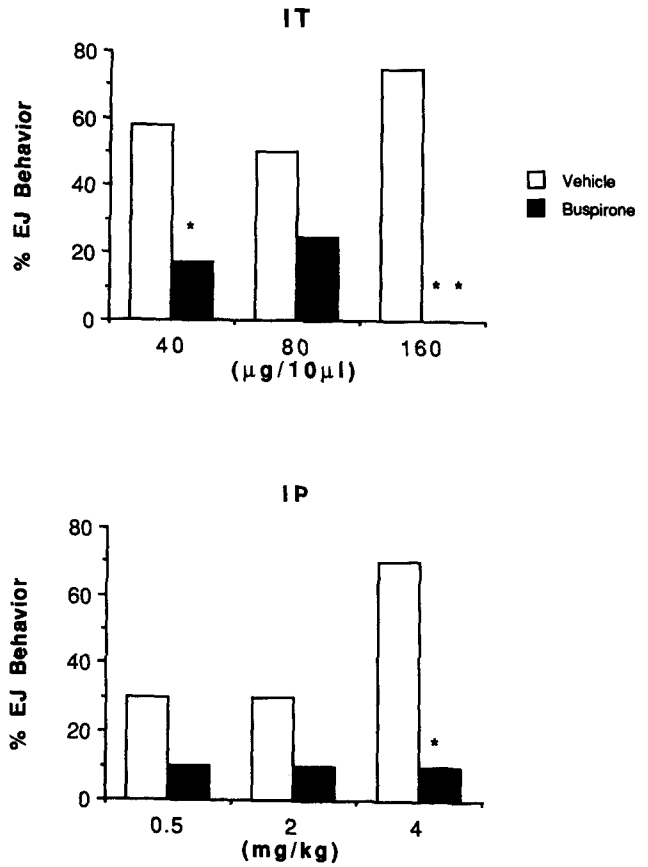

FIG. 1. Comparison of the effects of intrathecal (IT) and intraperitoneal (IP) administration of buspirone on the percentage of rats displaying ex copula ejaculation during the genital reflex test: *$p<0.05$, **$p<0.01$ in this and subsequent figures.

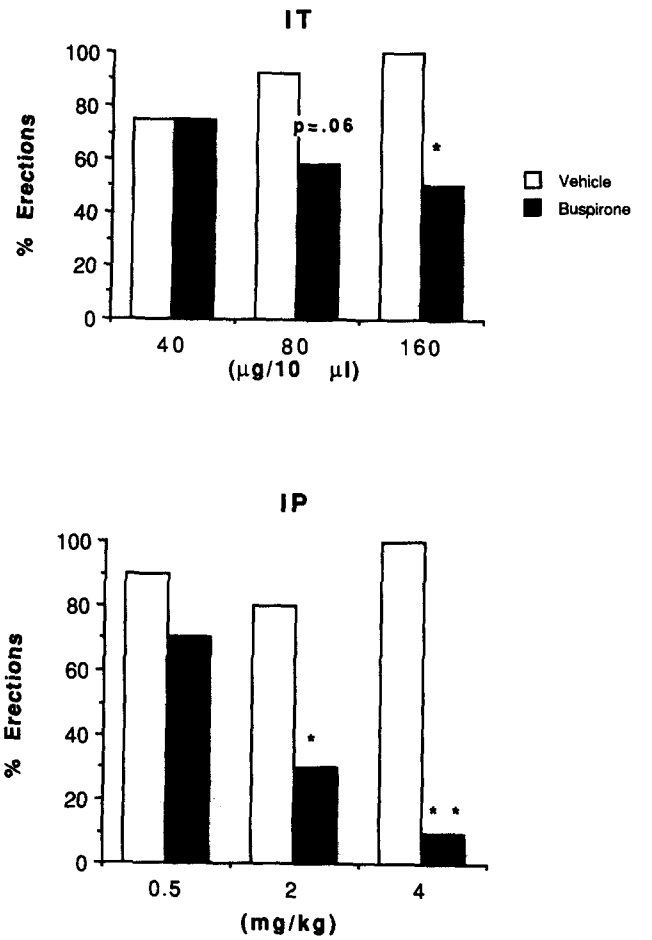

FIG. 2. Comparison of the effects of IT and IP buspirone on the percentage of rats showing erections in the genital reflex test.

ally, while the other animals were housed 2–4/cage. All were provided food and water ad lib and maintained in a 14:10 hour light:dark cycle (lights on 0500, lights off 1900). All genital reflex tests were conducted in the "lights on" period and all mating behavior tests during the "lights off" period.

Drugs

Buspirone hydrochloride was provided by Bristol-Myers Co. Immediately prior to testing, buspirone was dissolved in physiological saline (0.9% NaCl) and the drug or saline administered, by either the IT route or intraperitoneally (IP). Stimulus females used in mating tests were rendered receptive and proceptive via SC injections of (a) 150 μg estradiol benzoate 48 hr before and (b) 750 μg progesterone 4–6 hr before testing (both dissolved in 0.15 ml sesame oil).

Surgery

IT cannulation was performed according to the method of LoPachin *et al.* (12) with minor modifications as described earlier (11). Briefly, the operation involved threading a 12-cm catheter of PE-10 tubing through an incision made in the atlanto-occipital membrane and into the subarachnoid space; gently pushing the catheter caudally until it encountered the lumbar enlargement of the spinal cord. Rats were anesthetized with Avertin (tribromoethanol; 1.0 ml/100 g b.wt.). Cannulated rats were used repeatedly, each animal serving as its own control. Twenty-two catheterized rats survived the surgery without any motor, sensory or other impairment and were utilized. The most common limiting factor for continued use of an animal was deterioration and/or blockage of the external portion of the catheter tip.

Genital Reflex Test

This sequential test assesses separately, erection and associated reflexes and ejaculation (EJ) uncontaminated by sexual stimuli as described by Schnur *et al.* (20). First, the penis is checked for seminal material (ejaculate), immediately before injection. The rat is gently restrained in the supine position in a plastic cylinder and immediately observed during a 10-min period for the occurrence of EJ without penile sheath retraction. EJ was identified by rapid testicular retraction followed by seminal fluid expulsion. Ejaculate retained within the prepuce was verified on sheath retraction before the initiation of the erectile reflex test. The number of EJs and the latency of their deposition from test onset were recorded.

During the second observation period, erections and other penile reflexes were measured. According to Hart (9), the specific events recorded were erections, cups (erections with full flaring of the distal end of the glans), quick flips (abrupt dorsiflexion of the

TABLE 1

PENILE REFLEXES FOLLOWING IT AND IP ADMINISTRATION OF BUSPIRONE (MEDIANS)

	Erection Latency (min)	Number of Erections	Other Reflexes (Cups and Flips)
		IT	
40 μg busp.	6.25	10	1
saline	4.50	10	1
(n=7)			
80 μg busp.	10.77 (p=0.06)	13 (p=0.06)	0*
saline	3.50	13	4
(n=7)			
160 μg busp.	12.63*	7.5	1.5
saline	3.50	7.5	2
(n=6)			
		IP	
0.5 mg busp.	7.25*	8	0
saline	3.08	11	1
(n=7)			
2.0 mg busp.	9.00	8	0 ID
saline	2.33	16	1
(n=3)			
4.0 mg busp.	(3.25, 2.33) ID	(13, 7) ID	(0, 0) ID
saline	(7.75, 4.33)	(12, 15)	(0, 8)
(n=2)			

*p<0.05.
ID = insufficient data for statistical analysis.

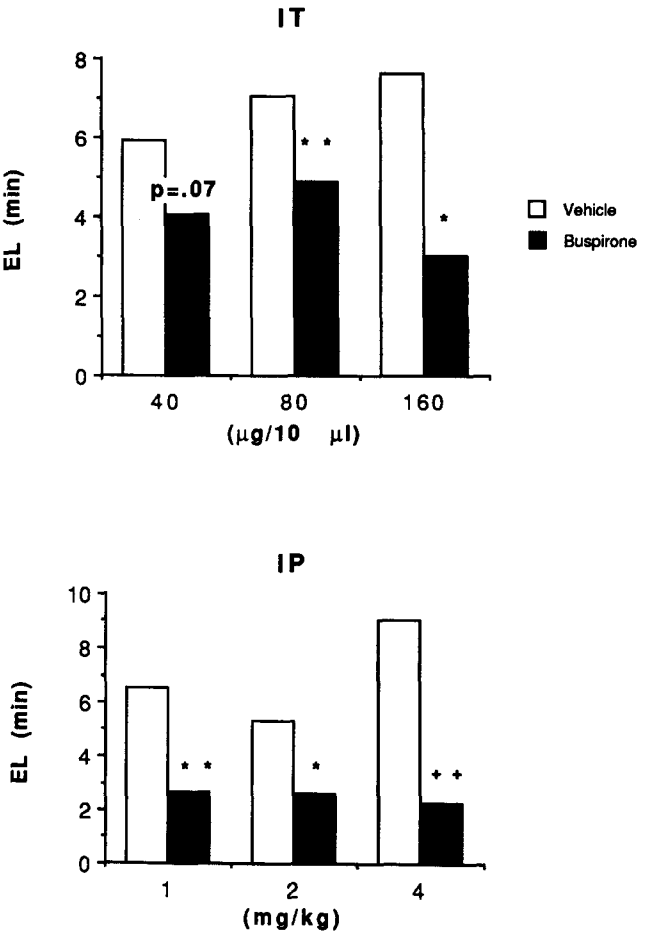

FIG. 3. Effects of IT and IP buspirone on ejaculation latency (EL). Data are presented as medians in this and subsequent figures: ^{++}p<0.001 in this and subsequent figures.

penis) and long flips (slower, more prolonged penile dorsiflexion). After the appearance of the initial erection, the animals were allowed to continue for an additional 10 min. If no erection occurred by 10 min, the test was terminated.

Experimental Design

A cross-over design was used, with each rat serving as its own control. IT doses of buspirone tested were 40, 80 and 160 μg/10 μl of saline or saline alone followed by a 10-μl saline flush. Infusions lasted over a 30–45 sec interval. Doses tested IP were 0.5, 2 and 4 mg/kg (b.wt.) buspirone. IT genital reflex tests had Ns of 12 at each dose while the IP tests had Ns of 10. There was a minimum of three weeks between different drug treatments. During week 1, half of the groups received the drug via the IT or IP route and the other half received IT or IP saline only. During week 2, all animals were tested without any treatment and during week 3, the half of the groups that received saline during week 1 then received drug (IT or IP) and the half that received drug during week 1 received saline. Week 4 was a no drug/saline test and the entire process was repeated again beginning with week 5 and using a different dose of drug.

Mating Behavior Test

A cross-over experimental design was used as above. Doses administered intrathecally were 40, 80 and 160 μg buspirone/10 μl with Ns of 10, 11 and 11, respectively. Intraperitoneal doses of buspirone were 1, 2 and 4 mg/kg with Ns of 11, 11 and 21, respectively. Immediately following treatments, the rats were placed in a semicircular observation cage, allowed to adapt to the testing arena and observed for occurrence of any unusual overt behaviors. Fifteen min later, a receptive female was introduced and observations of sexual behavior began. These continued until the first intromission of the second copulatory series or for 15 min if no intromission occurred or for 30 min after the first intromission, if no ejaculatory behavior occurred. The following parameters were recorded: mount latency (ML), the time from onset of test to the first mount with or without penile insertion; intromission latency (IL), time from the introduction of the female to the first intromission; ejaculation latency (EL), time from the first intromission to ejaculatory behavior; postejaculatory interval (PEI), time from ejaculatory behavior to the first intromission of the second copulatory series; mount frequency (MF), the number of mounts prior to ejaculatory behavior; intromission frequency (IF), the number of intromissions before ejaculatory behavior; and intercopulatory interval (ICI), the average time between intromissions (ICI = EL/IF+1), or if no ejaculatory behavior occurred, ICI = 30/IF. If after 7 min the male failed to intromit, the female was replaced.

Copulatory Plugs

An additional group of nine males was given 4 mg/kg bus-

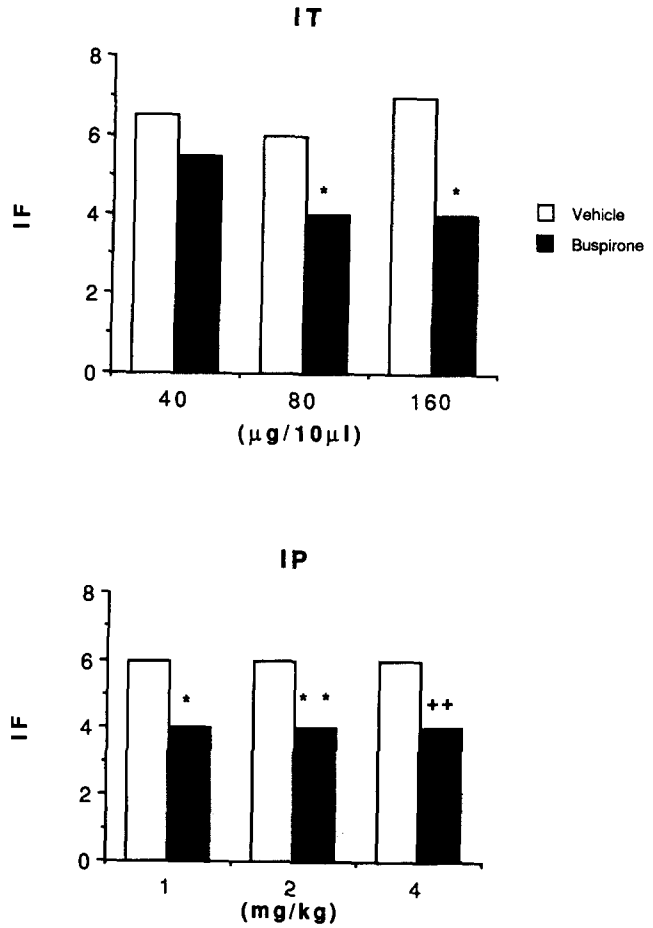

FIG. 4. Intromission frequency (IF) following IT and IP administration of buspirone.

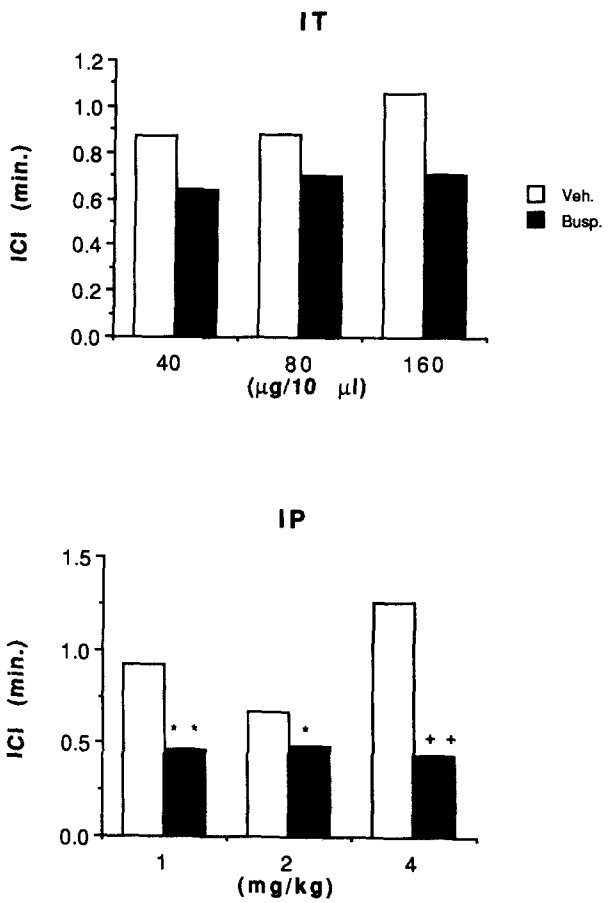

FIG. 5. The rate of mating behavior (ICI) after IT and IP buspirone treatment.

pirone IP, tested for mating behavior 15 min later and the copulatory plugs collected, as follows. After the PEI, the female was lightly anesthetized with ether and the vagina was examined for the presence of a copulatory plug. The vagina was douched with normal saline, the plug removed with forceps, allowed to air-dry for two weeks and weighed. This experiment followed the crossover design described above with each animal serving as its own control.

Autopsy

After completion of all intrathecal experiments, the cannulated rats were infused with 10 μl of thionin blue dye followed by a 10-μl saline flush. Fifteen minutes later, the animals were sacrificed using a 0.5 ml IP injection of T-61 euthanasia solution (Hoechst-Roussel Agri-Vet Co., Somerville, NJ) and the spinal cord dissected to locate the tip of the catheter and the presence of the dye.

Statistics

Except for the copulatory plug data, analysis was nonparametric. For analysis of percentages, the Binomial Test for significance of change was used, and for the behavioral parameters, the Wilcoxon-matched pairs signed-ranks test (21) was utilized. A paired *t*-test was used to analyze the copulatory plug data.

RESULTS

Autopsy

Dye injections and dissection of cannulated rats showed that the location of the catheter tips ranged from spinal level L1 to L4, with the majority of rats having placement at L3 (N = 11) or L2 (N = 8). Dye was found in the spinal segment of the catheter tip and, in most cases, one segment caudal to the tip.

Genital Reflex Test

As shown in Fig. 1 both IP and IT administration of buspirone drastically reduced the percentage of rats exhibiting ejaculation during this ex copula test. Following IP injection, there was a significant inhibition of EJ at the 4 mg/kg dose, while following IT infusion, significant inhibition was found at the 40 and 160 μg doses. Overall, the reductions ranged from 20–70% (IP) and 25–75% (IT).

Both IP and IT buspirone also significantly reduced the percentage of animals showing erections (Fig. 2), the change ranging from 20–80% (IP) and 0–50% (IT). Unlike the effect on EJ, the response for erections was dose-dependent. Additionally, rats that were positive in the erectile reflex test (Table 1), showed

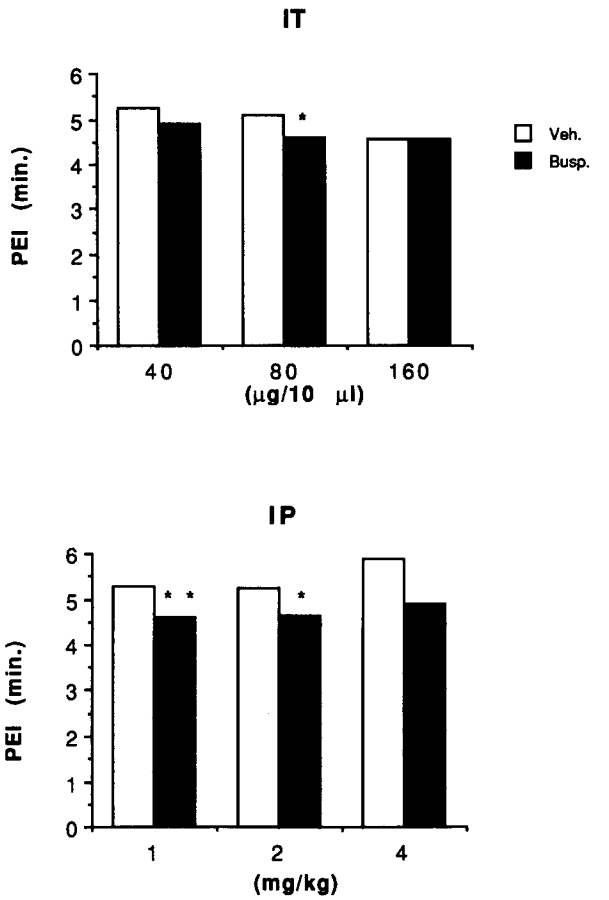

FIG. 6. The effects of IT and IP buspirone on the postejaculatory interval (PEI—sexual rearousal).

a significantly increased latency to the first erection at the IT dose of 160 μg and at the IP dose of 0.5 mg/kg. There was also a tendency towards a reduction of other reflexes, such as cups and flips, the frequency of which were significantly decreased at the 80 μg IT dose. Buspirone administered by either route did not significantly affect the number of erections displayed by animals showing erections although this response may be misleading, since the percentage of rats exhibiting this behavior was so low at the IP doses of 2 and 4 mg/kg (Fig. 2 and Table 1).

Mating Behavior Test

Both IP and IT administration of buspirone markedly affected various parameters of mating behavior. IT and IP buspirone significantly reduced EL and IF (Figs. 3 and 4), indicating a downward shift in the ejaculatory behavior threshold. The reductions in EL and IF did not show a dose-response effect following IP injection, but there was a trend towards a dose-response following IT infusion of the drug.

Intraperitoneal buspirone caused a significant decrease in ICI at all doses, demonstrating an increase in the rate of mating behavior, while IT infusion of the drug did not show this effect (Fig. 5). Moreover, the parameter for sexual rearousal (PEI) was significantly reduced following IP buspirone at all doses tested, while IT buspirone was ineffective except at the 80 μg dose (Fig. 6). Neither routes of drug administration had any significant effect on ML or IL (data not shown).

TABLE 2

MATING BEHAVIOR PARAMETERS FOR RATS USED TO COLLECT COPULATORY PLUGS FOLLOWING IP 4.0 mg/kg BUSPIRONE (MEDIANS)

	Parameter						
	MF (no)	IF (no)	ML (min)	IL (min)	EL (min)	PEI (min)	ICI (min)
Saline	3	9	0.1	0.2	5.10	5.15	0.46
Busp.	1	7*	0.1	0.1	1.95*	4.90	0.32*

*$p = <0.03$.

TABLE 3

HINDLIMB ABDUCTION AND FLATTENED BODY POSTURE DURING MATING BEHAVIOR FOLLOWING BUSPIRONE TREATMENT

	No. Displaying Behavior/Total	Percent Responding
IT (10 μl)		
40 μg buspirone	5/10	50.0
saline	0/10	0.0
80 μg buspirone	9/11	81.8†
saline	0/11	0.0
160 μg buspirone	9/11	81.8†
saline	0/11	0.0
IP (mg/kg)		
1 mg buspirone	6/11	54.5*
saline	0/11	0.0
2 mg buspirone	12/12	100†
saline	0/12	0.0
4 mg buspirone	20/21	95.2†
saline	0/21	0.0

*$p<0.05$, †$p<0.01$.

Copulatory Plug Collection

Behavioral data from the nine rats tested for copulatory plugs following IP administration of 4 mg/kg buspirone are presented in Table 2. This subset showed significant reduction in EL, IF and ICI. However, no significant effect of buspirone on plug weight was observed despite a trend towards reduction (saline: 36.8 ± 1.7 mg vs. buspirone: 29.6 ± 4.6 mg). Overall weights were 81.5% of controls, with individual values ranging from 2.8%–148.2% of controls.

Two of the three characteristics of the 5-HT syndrome (hindlimb abduction and flattened body posture) were observed during all mating behavior experiments. As shown in Table 3, the doses that elicited significant mating behavior changes showed the highest percentage of rats presenting with the syndrome. Even at the IT dose of 40 μg, 50% of the rats showed these behaviors. This low dose caused no significant change in EL or IF (Figs. 3 and 4).

DISCUSSION

DPAT and buspirone, two drugs with strong affinity for the

5-HT_{1A} receptor (10,16) have now been studied extensively with both IP and IT routes of administration. Comparisons with the present findings and those of DPAT (3, 11, 20) show a considerable concordance between the two drugs in terms of the specific profile of sexual effects in male rats. Moreover, both sites of administration, IP and IT, resulted in very similar effects.

Yet some diversity is found. The consistency of behavioral effects is stronger for ejaculatory than arousal measures. We speculate that the arousal component in these drugs is related to interaction with alpha$_2$-adrenoreceptors, though the affinity may not be extremely high (10). However, lisuride has very high affinity for both receptors (Peroutka, S. personal communication) and profoundly stimulates ejaculatory behavior and arousal (1).

It is the case that some drugs which have affinity for the 5-HT_{1A} receptor do not suppress ejaculation ex copula. Thus, RDS-127 (6–8, 22) shows a powerful ejaculatory behavior threshold reduction effect while ejaculation ex copula is stimulated. This drug is a dopaminergic (DA) agonist and other DA agonists such as apomorphine and quinelorane also stimulate ejaculation ex copula (4,5). However, 5-methoxy-N-N-dimethyltryptamine (MDMT), a 5-HT_{1A} and 5-HT_2 agonist, has this effect too, yet this drug is not known to have dopaminergic activity. Both RDS-127 and MDMT, moreover, show this effect in spinally sectioned animals (13,22).

It should also be noted that while IT buspirone showed trends towards a reduction in arousal as reflected in a lowered ICI, significant effects were only obtained following systemic treatment. IT DPAT, however, was effective in decreasing this measure (11). In contrast, IT buspirone significantly decreased PEI (a measure of rearousal) and IT DPAT did not. These results suggest that the more powerful sexual arousal effects seen following systemic 5-HT_{1A} agonist treatment probably occur at a cerebral locus(i). Nevertheless, in copula ejaculatory and to some degree, arousal behavior are stimulated via the lower spinal cord. One can only assume, then, that both these parts of the CNS regulate these particular behaviors, no doubt by interactive communication.

ACKNOWLEDGEMENTS

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